



(51) International Patent Classification:

C12N 9/12 (2006.01) *C12N 15/63* (2006.01)
C12N 15/54 (2006.01)

(21) International Application Number:

PCT/KR2012/000894

(22) International Filing Date:

7 February 2012 (07.02.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

10-2011-0011639 9 February 2011 (09.02.2011) KR

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH,

[Continued on next page]

(54) Title: REVERSE TRANSCRIPTASE HAVING IMPROVED THERMOSTABILITY

[Fig. 1]

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1 mlniedehrl hetskepdvs lqstwldefp qawaetggny lavraqplii plkatstpvv
61 ikqyymqsea rlgikphiqr lldqgilvpc qspwmtpllp vkkpgtndyr pvqdlrevnk
    →L
121 rvedihptvp apynllaglp pshqywtvld lkdaaffclrl hptsqplfaf ewrdpangis
181 gqitvtrlpq gfknsptlfd ealhrdladf riqhpdllil qyvddlllaa tseldcqqgt
241 rallqtignl gyrasakkaq icqkqvkyig yllkeqgrwl tearketvms qptpktprql
    →L          →Q
301 reflytagfc rlwipgfaem aaplypltkk gtlfmwydpq qkayqeikga lltapaalglp
    →L          →M
361 dlthpfcelfv dekgqyakgv ltqklqpmxz pvaylekklid pvaagvypcl zwvaaiavlt
    →E
421 kdagkitmgq plvilaphav calvkppdx wlsnarmthy qallldtdrv qfsgpvvalop
    →Y          →F
481 atllplpeeg lqhnclidila eahgtzpdlt dqplpdadht wytdqssllq eggrkagaav
541 tteteviwak alpagsaqr acialltgal kmaeqkkluv ytdsryafat ahihgeiyxr
601 rglltsegke iknkdeilal lkalfipkrl siihpcghqk ghsaaargnr madqaarkaa
661 itetpdtstl li

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(57) Abstract: The present invention relates to a reverse transcriptase having improved thermostability, more precisely a mutant reverse transcriptase with improved thermostability by substitution of one or more amino acids selected from the group consisting of the 63rd glutamine (Q63), the 264th lysine (K264), the 295th lysine (K295), the 306th threonine (T306), the 346th glutamic acid (E346), the 408th proline (P408), the 438th histidine (H438), and the 454th asparagin (N454) of the amino acid sequence of M-MLV originated reverse transcriptase represented by SEQ. ID. NO: 1 with other amino acids. The mutant reverse transcriptase of the present invention demonstrates excellent thermostability, compared with the wild type reverse transcriptase. Therefore, it is advantageous to obtain the target cDNA with stable reverse transcription activity even in the presence of RNA that can form the stable secondary structure at a high temperature.



GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*

— *with information concerning authorization of rectification of an obvious mistake under Rule 91.3 (b) (Rule 48.2(i))*

(88) Date of publication of the international search report:

20 December 2012

(15) Information about Correction:

Previous Correction:

see Notice of 1 November 2012

A. CLASSIFICATION OF SUBJECT MATTER*C12N 9/12(2006.01)i, C12N 15/54(2006.01)i, C12N 15/63(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 9/12; C12N 9/99; C12N 15/66; C40B 50/06; C12N 9/00; C12Q 1/68; C12N 9/22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: reverse transcriptase, MMLV, thermal stability, mutant.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002-0090618 A1 (MICHAEL D. SMITH et al.) 11 July 2002 See abstract; claims 2, 9, 10, 30-33, 48.	1-11
X	US 2008-0227661 A1 (HOLLY HOGREFE et al.) 18 September 2008 See abstract; claims 1-3, 9, 10, 25-27.	1-11
A	US 2003-0003452 A1 (ROBERT JASON POTTER et al.) 02 January 2003 See the whole document.	1-11
A	US 2007-0202529 A1 (CHRISTIAN E. GRUBER et al.) 30 August 2007 See the whole document.	1-11
A	US 06140086 A (DONNA K. FOX et al.) 31 October 2000 See the whole document.	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 SEPTEMBER 2012 (27.09.2012)

Date of mailing of the international search report

27 SEPTEMBER 2012 (27.09.2012)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
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Facsimile No. 82-42-472-7140

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YOO Min Jeong

Telephone No. 82-42-481-3463



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2012/000894

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

a. a sequence listing filed or furnished

- ☐ on paper
☒ in electronic form

b. time of filing or furnishing

- ☒ contained in the international application as filed
☐ filed together with the international application in electronic form
☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2012/000894

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002-0090618 A1	11.07.2002	AU 2001-247413 B2	25.08.2005
		AU 2001-47413 A1	24.09.2001
		AU 2001-71270 A1	11.12.2001
		AU 2003-274973 A1	30.04.2004
		CA 2401417 A1	20.09.2001
		CA 2406939 A1	06.12.2001
		CN 100390275 C0	28.05.2008
		CN 1422336 A0	04.06.2003
		CN 1430670 A0	16.07.2003
		EP 1266024 A1	18.12.2002
		EP 1266024 A4	30.07.2003
		EP 1290149 A1	12.03.2003
		EP 1290149 A4	17.03.2004
		EP 1554377 A2	20.07.2005
		EP 2295551 A1	16.03.2011
		EP 2325303 A2	25.05.2011
		EP 2325303 A3	17.08.2011
		JP 04-634799 B2	26.11.2010
		JP 2003-526378 A	09.09.2003
		JP 2004-511211 A	15.04.2004
		JP 2005-538719 A	22.12.2005
		US 2003-0003452 A1	02.01.2003
		US 2004-0209276 A1	21.10.2004
		US 2006-0094050 A1	04.05.2006
		US 2006-0211103 A1	21.09.2006
		US 2007-0141592 A1	21.06.2007
		US 2010-0015683 A1	21.01.2010
		US 2011-0081704 A1	07.04.2011
		US 2011-0165651 A1	07.07.2011
		US 7056716 B2	06.06.2006
		US 7078208 B2	18.07.2006
		WO 01-68895 A1	20.09.2001
		WO 01-92500 A1	06.12.2001
		WO 2004-024749 A2	25.03.2004
US 2008-0227661 A1	18.09.2008	None	
US 2003-0003452 A1	02.01.2003	AU 2001-247413 B2	25.08.2005
		AU 2001-47413 A1	24.09.2001
		AU 2001-71270 A1	11.12.2001
		AU 2003-274973 A1	30.04.2004
		CA 2401417 A1	20.09.2001
		CA 2406939 A1	06.12.2001
		CN 100390275 C0	28.05.2008
		CN 1422336 A0	04.06.2003
		CN 1430670 A0	16.07.2003
		EP 1266024 A1	18.12.2002
		EP 1266024 A4	30.07.2003
		EP 1290149 A1	12.03.2003

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2012/000894

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		EP 1290149 A4	17.03.2004
		EP 1554377 A2	20.07.2005
		EP 2295551 A1	16.03.2011
		EP 2325303 A2	25.05.2011
		EP 2325303 A3	17.08.2011
		JP 04-634799 B2	26.11.2010
		JP 2003-526378 A	09.09.2003
		JP 2004-511211 A	15.04.2004
		JP 2005-538719 A	22.12.2005
		US 2002-0090618 A1	11.07.2002
		US 2004-0209276 A1	21.10.2004
		US 2006-0094050 A1	04.05.2006
		US 2006-0211103 A1	21.09.2006
		US 2007-0141592 A1	21.06.2007
		US 2010-0015683 A1	21.01.2010
		US 2011-0081704 A1	07.04.2011
		US 2011-0165651 A1	07.07.2011
		US 7056716 B2	06.06.2006
		US 7078208 B2	18.07.2006
		WO 01-68895 A1	20.09.2001
		WO 01-92500 A1	06.12.2001
		WO 2004-024749 A2	25.03.2004
US 2007-0202529 A1	30.08.2007	AU 2000-50294 A1	12.12.2000
		AU 2000-50294 B2	26.08.2004
		CA 2374515 A1	30.11.2000
		EP 1185711 A1	13.03.2002
		EP 1185711 A4	18.09.2002
		JP 2003-500070 A	07.01.2003
		US 2003-0190661 A1	09.10.2003
		US 2006-0127936 A1	15.06.2006
		US 2008-0227107 A1	18.09.2008
		US 2009-0176242 A1	09.07.2009
		US 2010-0168408 A1	01.07.2010
		US 6589737 B1	08.07.2003
		US 7037661 B2	02.05.2006
		WO 00-71757 A1	30.11.2000
US 06140086 A	31.10.2000	CA 2339791 A1	25.02.1999
		CA 2339791 C	23.12.2008
		US 2005-0123905 A1	09.06.2005
		US 2011-0111464 A1	12.05.2011
		US 7736874 B2	15.06.2010
		WO 99-09197 A1	25.02.1999